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ORIGINAL ARTICLE

Altered lysophosphatidic acid (LPA) receptor expression during hepatic regeneration in a mouse model of partial hepatectomy

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Abstract

Background: Hepatic regeneration requires coordinated signal transduction for efficient restoration of functional liver mass. This study sought to determine changes in lysophosphatidic acid (LPA) and LPA receptor (LPAR) 1–6 expression in regenerating liver following two-thirds partial hepatectomy (PHx).

Methods: Liver tissue and blood were collected from male C57BL/6 mice following PHx. Circulating LPA was measured by enzyme-linked immunosorbent assay (ELISA) and hepatic LPAR mRNA and protein expression were determined.

Results: Circulating LPA increased 72 h after PHx and remained significantly elevated for up to 7 days post-PHx. Analysis of LPAR expression after PHx demonstrated significant increases in LPAR1, LPAR3 and LPAR6 mRNA and protein in a time-dependent manner for up to 7 days post-PHx. Conversely, LPAR2, LPAR4 and LPAR5 mRNA were barely detected in normal liver and did not significantly change after PHx. Changes in LPAR1 expression were confined to non-parenchymal cells following PHx.

Conclusions: Liver regeneration following PHx is associated with significant changes in circulating LPA and hepatic LPAR1, LPAR3 and LPAR6 expression in a time- and cell-dependent manner. Furthermore, changes in LPA–LPAR post-PHx occur after the first round of hepatocyte division is complete.

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Introduction

Liver regeneration is the process by which functional hepatic tissue is restored following damage or loss in liver mass.[1–3] In the clinical setting this is most commonly observed following the resection of a hepatic tumour or after a repair following trauma.[2,3] In the laboratory these clinical observations can be modelled, most commonly in mice and rats, by the administration of hepatotoxins (e.g. carbon tetrachloride) or by the surgical removal of one or more lobes of the liver in partial hepatectomy (PHx).[3,4] Although both approaches cause an effective decrease in functional liver mass, a two-thirds PHx, in which three of the five lobes of the (rodent) liver are resected, is often preferred because it facilitates reproducibility, accuracy of timing of events, and the absence of tissue damage to the remaining lobes.[4]

Following two-thirds PHx, hepatocytes are the first cells of the liver to undergo DNA synthesis/replication within 24–36 h, during which the liver approximately doubles in size. This is followed by a second round of DNA synthesis/replication to establish the pre-PHx hepatocyte population. The repopulation of other hepatic cell populations, such as the biliary epithelium and endothelial cells, typically occurs 2–3 days after PHx and 12–24 h after the first round of hepatocyte proliferation.[3–5] Regardless of the insult leading to the reduction in functional mass, the regeneration process requires a highly coordinated processing of cytokine, growth factor and metabolic networks.[5] These mechanisms are critical during the different stages of regeneration and range from detecting decreases in functional hepatic mass, through coordinated cell division and repopulation, and the orientation and restoration of cell phenotype function.[2,6]

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Heterotrimeric guanine nucleotide regulatory proteins (G-proteins) are ubiquitously expressed intracellular signalling molecules that act as intermediates which transduce extracellular signals to intracellular effectors via G-protein coupled receptors (GPCRs).[7] The intracellular pathways regulated following G-protein subunit dissociation depend on the specific α-subunit and/or βγ-dimer subunit compositions. For example, adenylyl cyclase activity is, for the most part, regulated by the balance in stimulatory (Gsα) and inhibitory (Giα) G-protein activation. This, in turn, regulates the levels of intracellular cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activity, an important regulator of cell function and gene transcription. Conversely, βγ-dimers associated (predominantly) with Giα-proteins are important regulators of mitogen-activated protein kinase (MAPK) signalling, a central regulator of cell proliferation.[8,9] Given the widespread distribution of G-proteins and their roles in regulating fundamental cell pathways and processes, it is of little surprise that Gi/Gs-proteins are involved in liver regeneration following PHx.[10,11] Somewhat more surprising is the relative lack of data identifying specific GPCRs that regulate intracellular G-protein activity during regeneration.

Lysophosphatidic acid (LPA) is a small (450-Da), ubiquitously expressed bioactive phospholipid[12,13] derived from phospholipid metabolism.[14] Lysophosphatidic acid is present in all eukaryotic tissue at low concentrations (pM–nM), and at higher concentrations in blood plasma (sub-μM).[13,14] Following synthesis, LPA interacts with specific cell surface receptors (LPARs) to regulate cell function. All LPARs identified to date are GPCRs and activate a range of G-protein subtypes following LPA–LPAR binding.[9,15] Signalling via LPA is involved in a diverse range of physiological and pathological events, including neoangiogenesis, neuronal growth, cardiovascular disease, fibrosis and cancer.[12,13] In liver biology and pathology, LPA is involved in mediating hepatic myofibroblast migration,[16] chemically induced liver injury,[17,18] and protecting against sepsis-induced liver damage.[18] However, the roles of specific LPARs in regulating events within the liver have been more difficult to define because LPARs are generally not well characterized[1–5] in human and rodent liver.[13]

In 2008 a novel non-endothelial differentiation gene (EDG) LPAR family member, LPAR6, was reported.[19] Because of the strong association between LPA and liver function, and the central role for G-protein signalling during hepatic regeneration, the present authors sought to determine whether PHx was associated with changes in LPA and LPAR-subtype expression and localization, including that of the most recently reported LPAR6 subtype, in a mouse model of liver regeneration after PHx.

Materials and methods

Institutional assurances

Male C57BL/6 mice (aged 8–10 weeks) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Studies were approved by the Institutional Animal Care and Use Committee, Carolinas Medical Center (Charlotte, NC, USA) and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Materials

Extraction and purification of RNA were performed using TRIzol (Invitrogen, Inc., Carlsbad, CA, USA). RQ1 DNase and the Improm-II reverse transcription system were purchased from Promega Corp. (Madison, WI, USA), and iQ SYBR Green Supermix was purchased from Bio-Rad Laboratories, Inc., (Hercules, CA, USA). Antibodies against proliferating cell nuclear antigen (PCNA) and LPAR6 were purchased from Abcam, Inc. (Cambridge, MA, USA), and antibodies against LPAR1 and LPAR3 were purchased from Novus Biologicals, Inc. (Littleton, CO, USA) and EMD Millipore Corp. (Billerica, MA, USA), respectively. An enzyme-linked immunosorbent assay (ELISA) to detect plasma LPA levels was purchased from Echelon Biosciences, Inc. (Salt Lake City, UT, USA).

Partial hepatectomy

A two-thirds PHx was performed in male mice as previously reported.[20] Briefly, mice were anaesthetized using isoflurane by inhalation and placed in a supine position on a warming pad. A midline incision was made and the upper abdomen opened to allow the falciform ligament to be divided to the level of the superior vena cava. The lobes to be resected (the left lobe followed by the median lobe) were gently lifted and a 4–0 silk suture placed underneath the lobe as proximal to the origin as possible. The suture was then tied at the base of the lobe and the lobe cut distal to the suture. Resected tissue was weighed, cut and snap frozen in liquid nitrogen or placed in neutral buffered formalin prior to processing for mRNA analysis or histology and immunohistochemistry (IHC). Mice received ~ 0.7 ml of intraperitoneal sterile 0.9% saline to correct for fluid loss and the abdominal muscle and skin were closed in two layers. At predetermined endpoints (12 h, 24 h, 48 h, 72 h, 96 h and 7 days post-PHx) mice were killed by exsanguination, blood collected by cardiac puncture and plasma prepared by centrifugation. Liver tissue was removed, grossly examined, weighed, and either snap frozen in liquid nitrogen or placed in neutral buffered formalin.[21]

Circulating LPA levels

Plasma was collected and analysed for circulating LPA levels using a commercially available ELISA according to the manufacturer's instructions.

Tissue LPAR mRNA expression

Total RNA was extracted from flash frozen liver tissue and analysed by quantitative reverse transcription polymerase chain reaction using 50 ng cDNA and gene-specific oligonucleotide primers (Table 1). Relative mRNA levels were calculated, normalized to β-2-microglobulin (*β2M*, housekeeping gene), and expressed as the fold change in expression for each LPAR subtype in regenerating liver samples relative to that in pair-matched liver tissue obtained at the time of resection. Liver tissue from sham-operated

Table 1 Forward and reverse primer sequences for lysophosphatidic acid receptors (LPARs) 1, 3, and 6 used for quantitative reverse transcription polymerase chain reaction analysis

Gene	Forward primer	Reverse primer
LPAR1	5′ CTGCCTCTACTTCCAGCCCTGTAA 3′	5′ TGCTCACTGTGTTCCATTCTGTGG 3′
LPAR3	5′ CCACTTTCCCTTCTACTACCTGCT 3′	5′ GACGGTCAACGTTTTCGACACC 3′
LPAR6	5′ GATCACTCTCTGCATCGCTGTTTC 3′	5′ CCCTGAACTTCAGAGAACCTGGAG 3′
β2M	5′ CTCGGTGACCCTGGTCTTTCTGGTG 3′	5′ TCTCCGGTGGGTGGCGTGAGTATA 3′

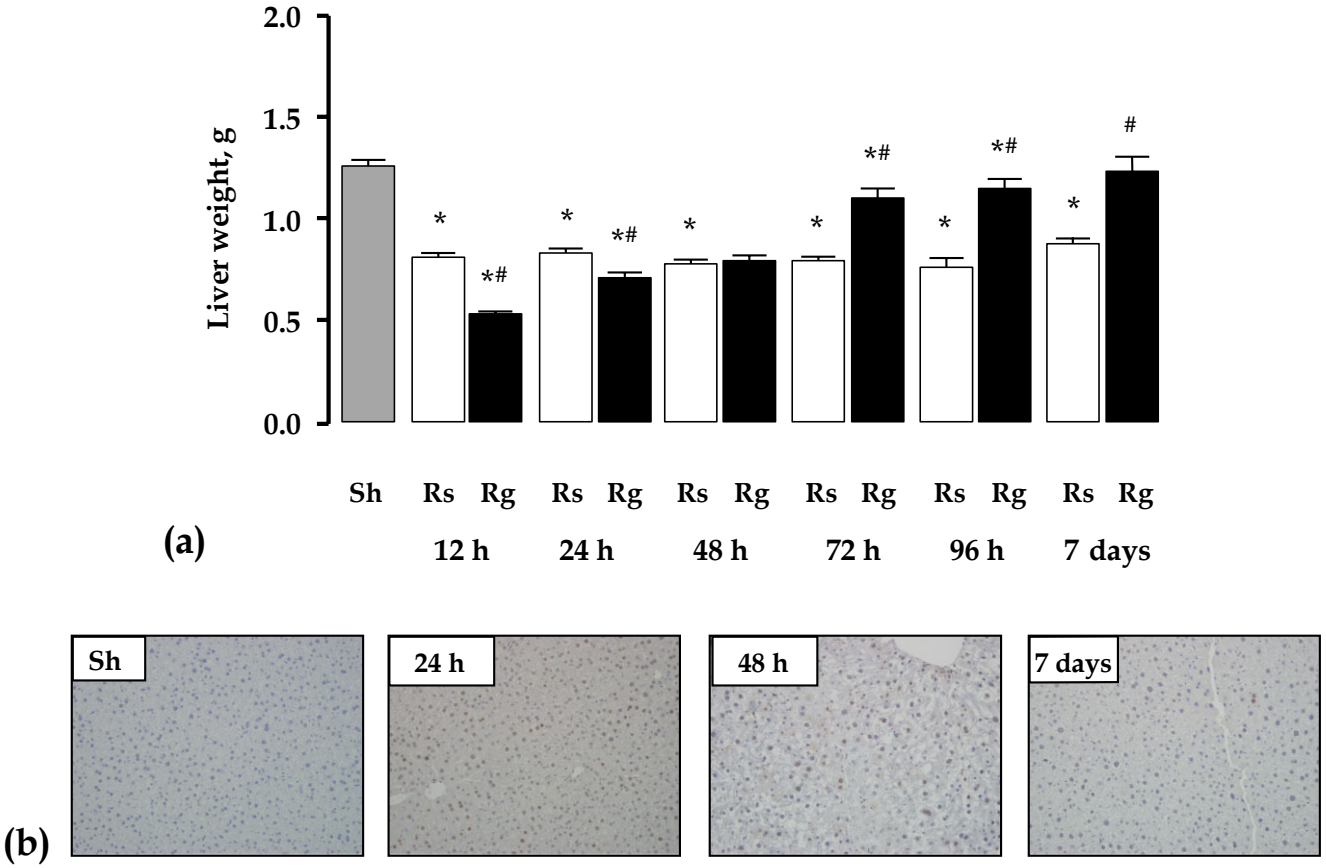

Figure 1 Hepatic regeneration following two-thirds partial hepatectomy (PHx) in male C57BL/6 mice. (a) Liver weights were obtained from sham-operated (Sh) mice or following resection of the left and median hepatic lobes (Rs). Remnant/regenerating (Rg) liver weights were obtained at 12 h, 24 h, 48 h, 72 h, 96 h and 7 days post-PHx. *, $P < 0.05$ versus sham liver weight; #, $P < 0.05$ Rs versus Rg; $n = 5$ animals per time-point. (b) Representative immunohistochemistry images using an antibody specific against proliferating cell nuclear antigen (PCNA; brown staining) in sections from Sh and Rg tissue at 24 h, 48 h and 7 days post PHx

animals was collected at 12 h post-surgery and analysed for LPAR1–6 mRNA expression and findings were compared with those in normal liver obtained at the time of resection. To directly compare LPAR1, LPAR3 and LPAR6 mRNA expression, data were normalized to *β2M* expression.[21]

Tissue histology and LPAR protein expression

Liver lobules were sectioned (4 μm) and stained with haematoxylin and eosin (H&E); representative sections were examined microscopically (original magnification: ×100). Hepatic LPAR1, LPAR3 and LPAR6 expression and localization in control (resected) and regenerating liver were established by IHC as previously reported.[21] Anti-LPAR1 was used at a dilution of 1:100; anti-LPAR3 was used at a dilution of 1:200, and anti-LPAR6 was used at a dilution of 1:500. Five random fields per slide were viewed and blind-scored using a scale of 0–3 on which 0 represents the absence of detectable stain.

Statistics

Data are presented as the mean ± standard error of the mean (SEM), as appropriate. Statistical analysis was performed using GraphPad Prism Version 5.0b (GraphPad Software, Inc., La Jolla, CA, USA). Group-wide analysis was performed by one-way analysis of variance (ANOVA). Pairwise combinations within a group were analysed by paired Student's *t*-test. A *P*-value of <0.05 was considered to indicate statistical significance.

Results

Liver regeneration following two-thirds PHx

Resection of the left and median hepatic lobes resulted in a decrease in liver weight of approximately 65% compared with control (sham-operated) liver weight [mean resected liver weight: 0.81 ± 0.01 g (n = 30 samples); mean sham-operated liver weight: 1.24 ± 0.05 g (n = 4 samples); $P < 0.05$] (Fig. 1a). There was no significant difference in mean animal weight (24.3 ± 0.3 g, n = 30), or in the weight of resected liver tissue among animals randomized to the six respective time-points (n = 5 animals per time-point) (Fig. 1a). Prior to death, four animals appeared jaundiced and exhibited signs of distress, which led to their removal from the study and replacement with additional animals. With the exception of these four mice, H&E staining revealed normal hepatic architecture and histology consistent with regenerating liver tissue (data not shown). Immunohistochemical analysis using an antibody against proliferating cell nuclear antigen (PCNA) confirmed hepatic regeneration: intense nuclear PCNA staining was detected at 24 h and 48 h post-PHx (Fig. 1b).

Partial hepatectomy leads to altered circulating LPA levels

No significant difference in circulating LPA was detected over the first 48 h in animals submitted to two-thirds PHx compared with sham-operated animals (n = 5 per group) (Fig. 2). Conversely, LPA increased significantly at 72 h post-PHx to 6.30 ± 0.67 μM compared with 3.58 ± 0.37 μM in sham-operated animals (n = 5 per group; $P < 0.05$) (Fig. 2) and remained significantly elevated in post-PHx liver compared with sham-operated liver for up to 7 days post-PHx (n = 5 per group; $P < 0.05$ at 96 h and 7 days) (Fig. 2).

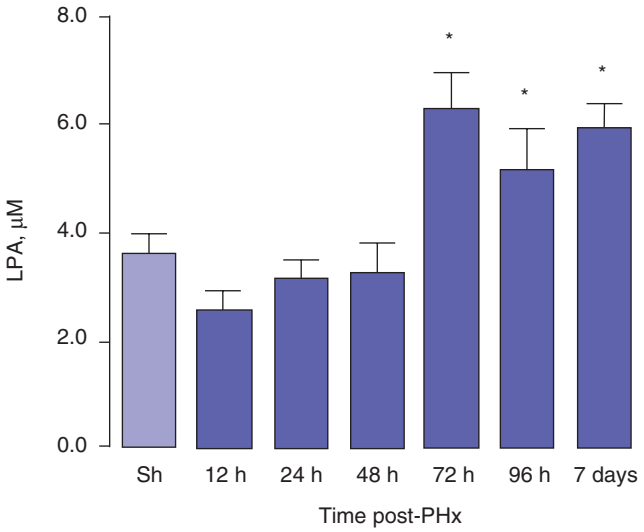

Figure 2 Partial hepatectomy (PHx) leads to increased circulating lysophosphatidic acid (LPA). Circulating LPA levels in sham-operated (Sh) mice or mice following resection of the left and median hepatic lobes. *, $P < 0.05$ versus sham-operated; n = 5 animals per time-point

Partial hepatectomy alters hepatic LPAR1, LPAR3 and LPAR6 mRNA expression

Quantitative reverse transcription polymerase chain reaction analysis demonstrated LPAR2, LPAR4 and LPAR5 mRNA was barely detectable, if at all, in normal liver, and did not significantly change following PHx (data not shown). By contrast, LPAR1, LPAR3 and LPAR6 mRNA was detected in sham-operated, resected and regenerating liver tissue, and expression changed in a time-dependent manner following PHx (Fig. 3a–c). Following resection, an initial peak in LPAR1 mRNA was detected at 12 h and 24 h, followed by a second increase at 72 h and 96 h in regenerating liver (n = 5 per time-point; $P < 0.05$ in regenerating liver versus pair-matched resected liver) (Fig. 3a). By contrast, LPAR3 mRNA expression increased sharply at 12 h, by approximately 10-fold, in regenerating liver before returning to levels that did not significantly differ from those in resected liver at the remainder of the time-points analysed (24 h to 7 days) (n = 5 per time-point; $P < 0.05$ at 12 h post-PHx compared with pair-matched resected liver) (Fig. 3b). Analysis of LPAR6 mRNA demonstrated a significant increase in regenerating liver within 12 h of PHx, and LPAR6 mRNA remained significantly elevated at the remaining time-points (24 h to 7 days) (n = 5 per time-point; $P < 0.05$, post-PHx tissue versus pair-matched resected liver) (Fig. 3c). No significant difference in LPAR1, LPAR3 or LPAR6 mRNA was detected between sham-operated and resected liver tissue (data not shown). A direct comparison of LPAR1, LPAR3 and LPAR6 levels demonstrated significantly greater expression of LPAR6 mRNA than LPAR1 mRNA or LPAR3 mRNA in both resected liver and regenerating liver at all time-points (n = 5 per time-point; $P < 0.05$ for LPAR6 mRNA versus LPAR1 and LPAR3 mRNA at 24 h) (Fig. 3d).

Partial hepatectomy alters hepatic LPAR1, LPAR3 and LPAR6 protein expression

No significant differences in LPAR1, LPAR3 or LPAR6 expression were detected between sham-operated tissue and liver tissue resected during PHx (data not shown). Analysis demonstrated relatively low expression of LPAR1 in normal (sham-operated) liver tissue (Fig. 4a, c). Following PHx, LPAR1 expression increased significantly in regenerating tissue analysed at 48 h and remained elevated at the remainder of the time-points analysed (n = 5 per time-point; $P < 0.05$ at 48 h, 72 h, 96 h and 7 days in PHx

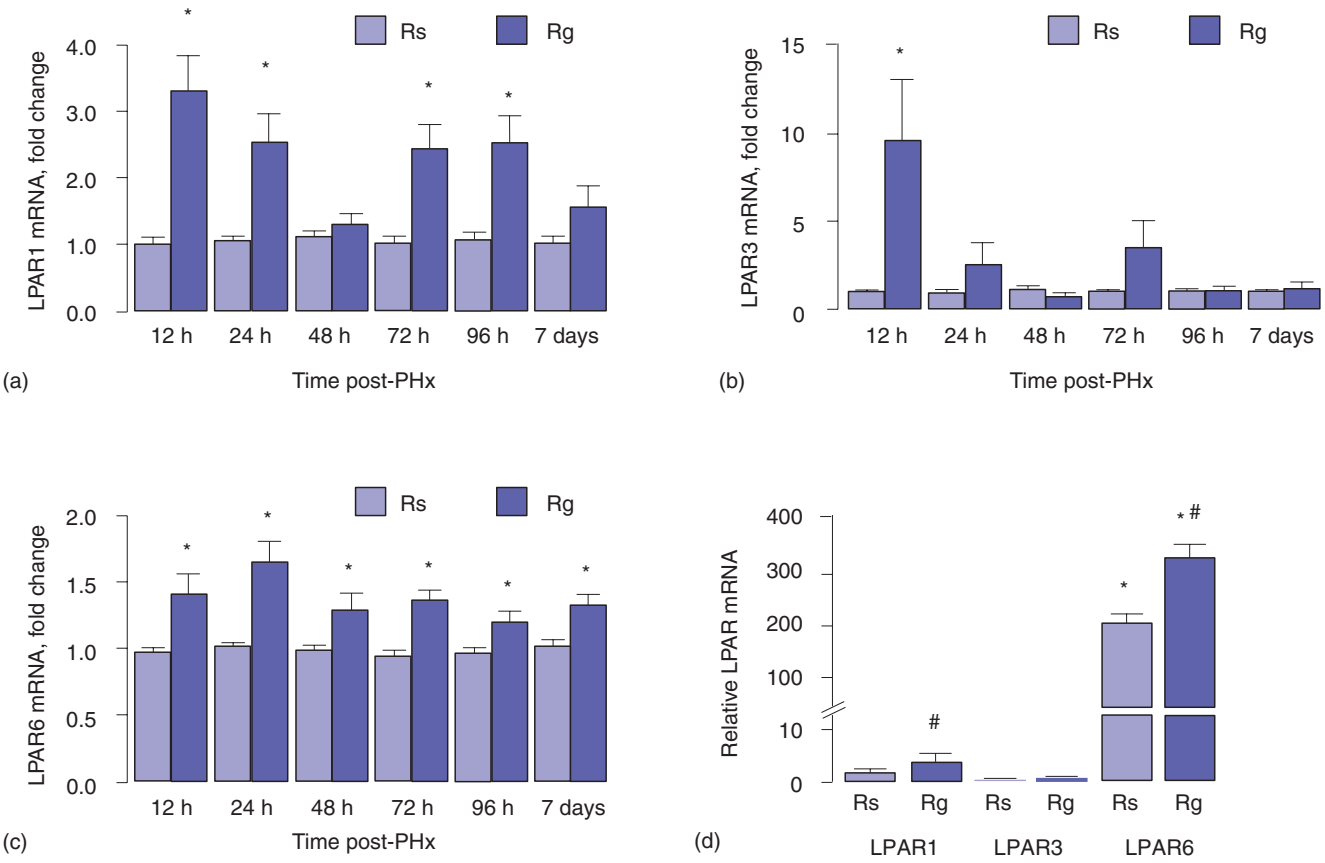

Figure 3 Partial hepatectomy (PHx) leads to altered lysophosphatidic acid receptor (LPAR) mRNA expression. Fold change in expression of (a) LPAR1, (b) LPAR3 and (c) LPAR6 mRNA in regenerating liver tissue (Rg) and pair-matched resected liver (Rs) following two-thirds PHx. *, $P < 0.05$ Rg versus Rs; $n = 5$ animals per time-point. (d) Relative expression of LPAR1, LPAR3 and LPAR6 mRNA in Rs and Rg liver tissue at 24 h post-PHx. *, $P < 0.05$ LPAR6 mRNA versus corresponding (Rs and Rg) LPAR1 and LPAR3 mRNA expression; #, $P < 0.05$ Rg versus Rs; $n = 5$ animals per time-point

versus sham-operated liver tissue) (Fig. 4c). Of particular note, when LPAR1 expression was increased, detection appeared localized to non-parenchymal cells (Fig. 4b, grey arrows). As with LPAR1, LPAR3 was also relatively low in normal liver tissue and increased at 48 h post-PHx ($n = 5$ per time-point; $P < 0.05$ at 48 h, 72 h, 96 h and 7 days in PHx versus sham-operated animals) (Fig. 5a, c). Unlike LPAR1, LPAR3 appeared to be more widely distributed (Fig. 5a, b). Finally, analysis of LPAR6 protein demonstrated an initial modest, although significant, increase in expression within 12 h of PHx, before a second, more sustained increase in expression was detected at 48 h post-PHx ($n = 5$ per time-point; $P < 0.05$ at 12 h, 48 h, 72 h, 96 h and 7 days in PHx versus sham-operated animals) (Fig. 6a, c). As with LPAR3, LPAR6 staining was widely distributed (Fig. 6a, b).

Discussion

Hepatic regeneration following PHx requires complex, coordinated signalling events.[1–3,5] The aims of this study were to analyse changes in LPAR signalling during hepatic regeneration in an experimental mouse model, including that of the most recently characterized LPAR isoform, LPAR6. The present paper reports that the initiation of hepatic regeneration using a two-thirds PHx model led to significantly increased levels of circulating LPA and altered hepatic expression of LPAR1, LPAR3 and LPAR6. A role for LPA–LPAR signalling has been indicated in a range of hepatic disease states.[16–18,21–23] However, previously characterized LPAR subtypes (LPAR1–5) are expressed weakly, if at all, in the liver. Thus identification of LPAR6 in normal liver, and subsequent changes in expression during regeneration, may prove significant in furthering current understanding of the role of LPA signalling in liver physiology and pathology.

Several experimental approaches and rodent models of PHx have been described. Using a model of 65–70% (two-thirds) PHx, in which the left and median lobes were resected, the present group first demonstrated the technical reproducibility of resection and regeneration rates consistent with those reported by others. Analysis of circulating LPA following PHx demonstrated a brief

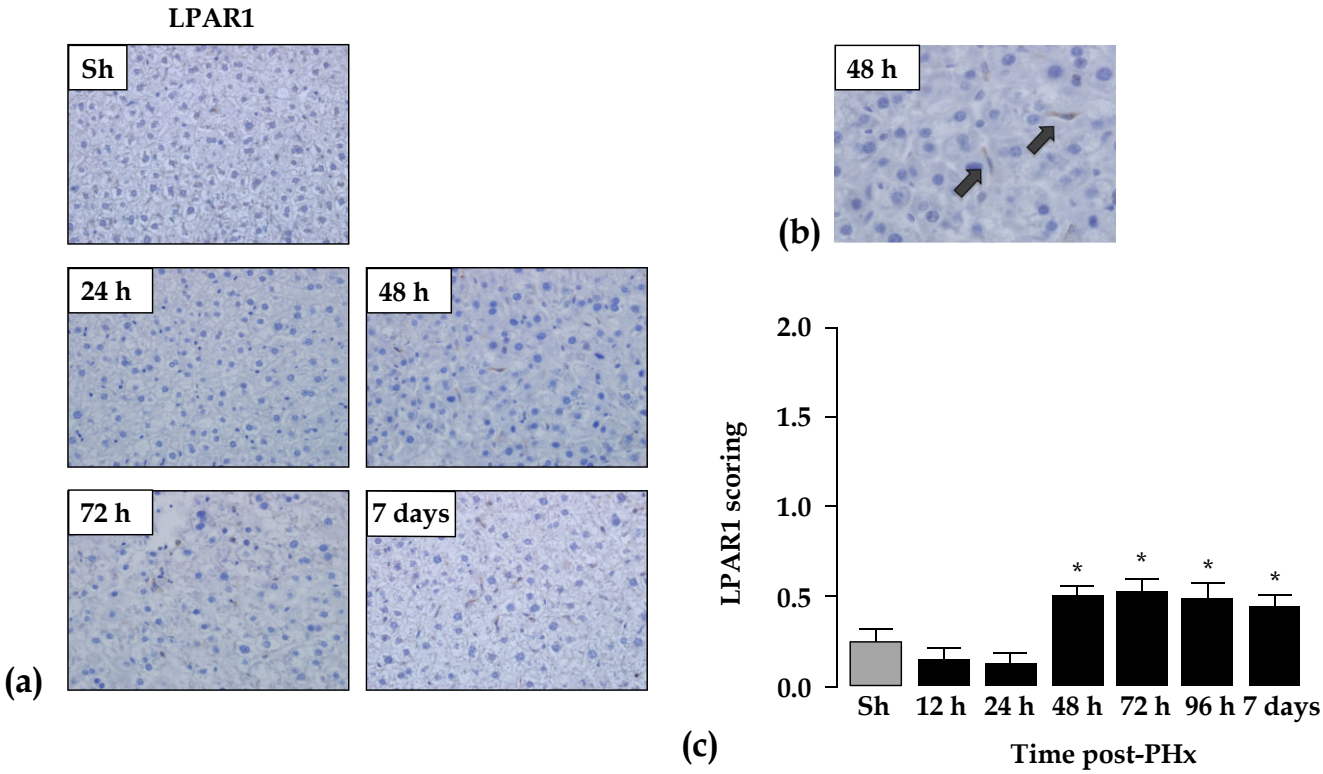

Figure 4 Partial hepatectomy (PHx) leads to altered lysophosphatidic acid receptor-1 (LPAR1) protein expression. (a) Representative immunohistochemistry (IHC) images of sham-operated (Sh) or regenerating (Rg) liver tissue following two-thirds PHx analysed for LPAR1 expression (×200). (b) Representative IHC image of LPAR1 expression in regenerating liver at 48 h after two-thirds PHx (×400). Grey arrows indicate non-parenchymal cell staining. (c) Representative fields (n = 5 per time-point) were blind scored on a scale of 0–3. *, $P < 0.05$ Rg versus Sh; n = 5 animals per time-point

drop in LPA (at 12 h), followed by a return to baseline values (at 24–48 h) and a subsequent significant increase from 72 h until the end of the experiment protocol (at 7 days). Using a rat model, Watanabe *et al.* reported plasma LPA increased in response to 70% PHx within 24 h.[24] A possible explanation for this discrepancy may lie in differences between the rat and mouse models of PHx because cell replication occurs earlier in rats than in mice. Alternatively, Watanabe *et al.* employed a colorimetric enzymatic cycling assay to detect LPA,[24] whereas the present authors employed a commercially available ELISA. This may be of particular significance for the detection of LPA because the term 'LPA' actually refers to a range of lipid-like substances formed following phospholipid metabolism. As such, a number of LPA species with widely differing biological properties can be formed.[12–14] Because the ELISA employed in the current study uses a single antibody to detect LPA, it may not be sensitive to detect other earlier changes in LPA subtype levels following PHx.[12,25]

Although it is possible that the ELISA employed was not sufficiently sensitive to detect changes in different LPA subtypes following PHx, other possibilities must also be considered. To date, the role of LPA signalling in liver biology and pathology has been ambiguous. Systemic LPA is reported to affect liver function and changes in hepatic LPA-dependent signalling are reported in response to physiological and pathological stressors. Conversely, although the liver appears to be a target for LPA activity, previously characterized LPARs 1–5 are weakly expressed or undetectable in rodent and human liver. In line with these earlier reports,[13,23] the present authors detected LPAR1 and LPAR3 mRNA and protein, albeit at relatively low levels, in normal mouse liver. By contrast, LPAR6 mRNA was abundantly detectable in normal liver (relative to LPAR1 and LPAR3 mRNA).

Following PHx, both LPAR1 mRNA and protein expression increased significantly. Albeit that the corresponding increases in LPAR1 protein were relatively modest, it was particularly interesting to note that LPAR1 staining was confined to non-parenchymal cells. Although the present study did not facilitate the further identification of specific cell populations [hepatic stellate cells (HSCs), endothelial cells, Kupffer cells], it is of interest to note that other investigators report LPA stimulates proliferation[26] and contraction[27] of HSCs *in vitro*. This may be of further relevance in view of the need to repopulate the liver with HSCs following regeneration, and the role of HSCs in supporting the regenerative process via the release of mitogens associated with hepatocyte growth, a process that requires a transient change in HSC phenotype to an

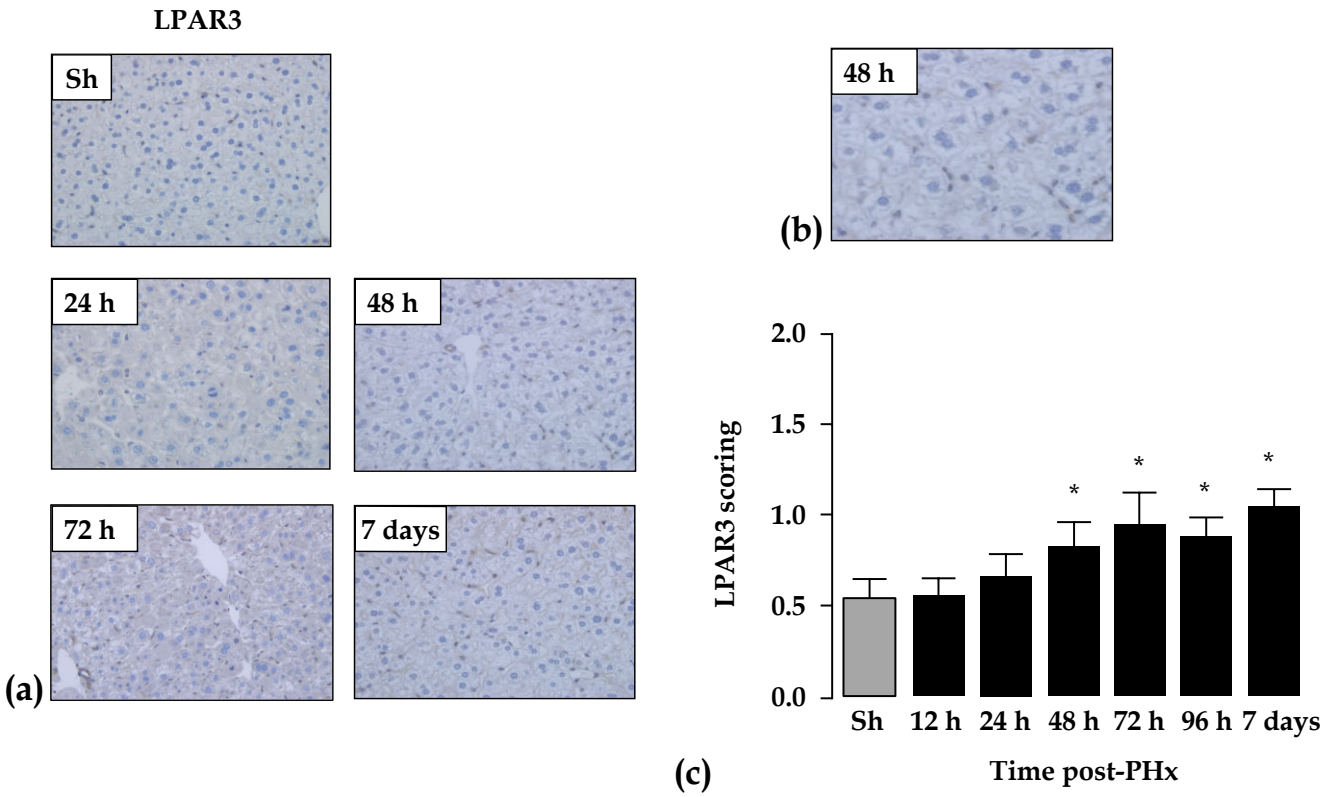

Figure 5 Partial hepatectomy (PHx) leads to altered lysophosphatidic acid receptor-3 (LPAR3) protein expression. (a) Representative immunohistochemistry (IHC) images of sham-operated (Sh) or regenerating (Rg) liver tissue following two-thirds PHx analysed for LPAR3 expression (×200). (b) Representative IHC image of LPAR3 expression in regenerating liver at 48 h following two-thirds PHx (×400). (c) Representative fields (n = 5 per time-point) were blind scored on a scale of 0–3. *, $P < 0.05$ Rg versus Sh; n = 5 animals per time-point

activated state.[28–30] Indeed, if cells staining for LPAR1 post-PHx are HSCs, this may explain why a biphasic change in LPAR1 mRNA occurs, in which the first increase (at 12–24 h) coincides with an HSC-dependent mitogen release and the initial round of hepatocyte proliferation, and the second increase (at 48 h to 7 days) is involved in HSC repopulation.

Analysis of LPAR3 identified an increase in mRNA expression within 12 h of PHx, followed by elevated protein expression at 48 h post-PHx. Although LPAR6 mRNA and protein also increased within 12 h, unlike LPAR3 the increases in LPAR6 expression were sustained at later time-points (48 h to 7 days post-PHx). Additionally, LPAR6 staining was more widespread than that observed for LPAR1 or LPAR3. This may be of particular significance in delineating a potential role for LPAR6-dependent signalling in experimental models of PHx. Following two-thirds PHx in mice, the initial round of hepatocyte proliferation peaks at ≈ 36 h, is followed by biliary epithelial cells and subsequently sinusoidal endothelial cells concomitant with a second round of hepatocyte repopulation.[1–3,5] Because the present data demonstrate that the most significant changes in LPA production and LPAR expression do not occur until ≥48 h post-PHx, it would seem fair to presume LPA–LPAR signalling is not a central mediator of initial hepatocyte repopulation. Rather, the current data suggest that it is more likely that LPA–LPAR6 signalling mediates proliferation of other hepatic cell populations or serves a function other than that of a mitogenic factor in the regenerating liver.

Following regeneration/repopulation, it is important that cells undergo differentiation to acquire the phenotype necessary to perform the functions demanded of them. For example, a basic physiological function of hepatocytes is the production, modification and secretion of bile. For this to occur following hepatocyte division, it is essential that not only are the biochemical and enzymatic processes needed to manufacture the components of bile are restored, but that the hepatocyte also orientates correctly within the sinusoid to form the basal (sinusoidal) and apical (canalicular) membranes required for bile secretion. Similarly, for repopulation to progress, it is necessary for basement membrane remodelling to occur to generate the space which dividing hepatocytes will occupy. Preliminary data indicate that LPAR6 activation leads to intracellular Rho activation or cAMP–PKA signalling. Although this aspect requires more investigation, it is interesting to note that Rho activation is widely implicated during cell migration and mitogenesis,[31] and cAMP–PKA signalling is important to the process of hepatocyte orientation[32,33] and to

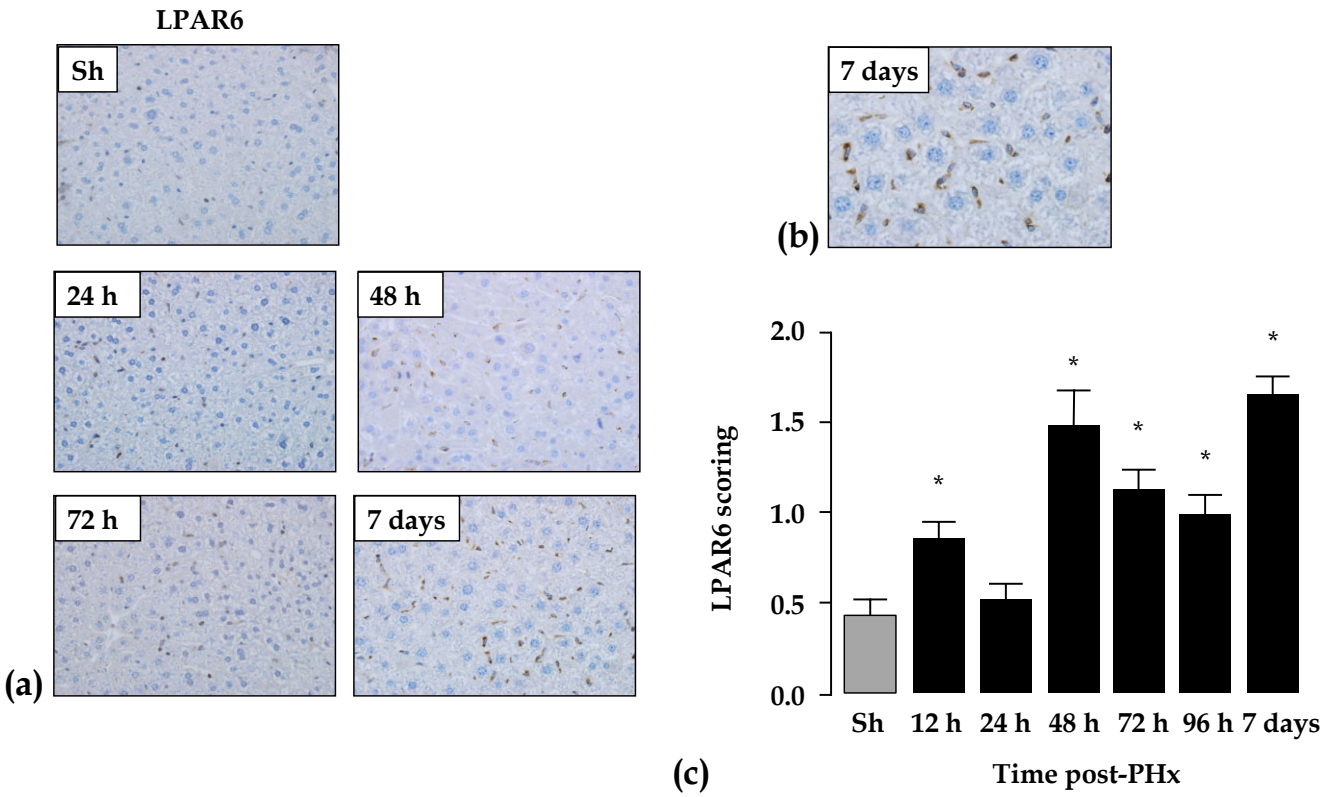

Figure 6 Partial hepatectomy (PHx) leads to altered lysophosphatidic acid receptor-6 (LPAR6) protein expression. (a) Representative immunohistochemistry (IHC) images of sham-operated (Sh) or regenerating (Rg) liver tissue following two-thirds PHx analysed for LPAR6 expression (×200). (b) Representative IHC image of LPAR6 expression in regenerating liver at 7 days following two-thirds PHx (×400). (c) Representative fields (n = 5 per time-point) were blind scored on a scale of 0–3. *, $P < 0.05$ Rg versus Sh; n = 5 animals per time-point

water channel (aquaporin) localization within the basolateral and canalicular membranes during bile production.[34–36]

The data presented herein suggest further investigation is warranted to delineate the role of LPA–LPAR signalling during hepatic growth and the restoration of cell function in the regenerating liver. In such investigations it may prove equally important to consider other potential functions for LPA–LPAR signalling during the restoration of functional liver mass. For example, the (relatively) late changes in LPA–LPAR signalling that occur following PHx may indicate LPA signalling plays a role in other important events, such as hepatic vascular remodelling and/or the re-establishment of the three-dimensional architecture of the sinusoid that is critical to the restoration of liver function. Indeed, a significant body of literature has addressed the importance of changes in blood volume and blood flow during regeneration following PHx, and the impact of these factors in stimulating and regulating the regenerative process.

Conclusions

The present data demonstrate that hepatic regeneration increases circulating LPA and LPAR1, LPAR3 and LPAR6 expression in a mouse model of PHx. Of particular note, changes in LPA and LPAR expression were not detected until after the first round of hepatocyte proliferation was complete, which suggests that LPA may be involved in the proliferation of other hepatic cell types and/or the restoration of cell function following initial hepatocyte proliferation.

Conflicts of interest

None declared.

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